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Genetic Markers of ADHD-Related Variations in Intracranial Volume

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Abstract

Objective: Attention deficit hyperactivity disorder (ADHD) is a common and highly heritable neurodevelopmental disorder with a complex pathophysiology. Intracranial volume (ICV) and volumes of the nucleus accumbens, amygdala, caudate nucleus, hippocampus, and putamen are smaller in people with ADHD compared with healthy individuals. The authors investigated the overlap between common genetic variation associated with ADHD risk and these brain volume measures to identify underlying biological processes contributing to the disorder.

Methods: The authors combined genome-wide association results from the largest available studies of ADHD (N=55,374) and brain volumes (N=11,221–24,704), using a set of complementary methods to investigate overlap at the level of global common variant genetic architecture and at the single variant level.

Results: Analyses revealed a significant negative genetic correlation between ADHD and ICV (r_g= −20.22). Meta-analysis of single variants revealed two significant loci of interest associated with both ADHD risk and ICV; four additional loci were identified for ADHD and volumes of the amygdala, caudate nucleus, and putamen. Exploratory gene-based and gene-set analyses in the ADHD-ICV meta-analytic data showed association with variation in neurite outgrowth-related genes.

Conclusions: This is the first genome-wide study to show significant genetic overlap between brain volume measures and ADHD, both on the global and the single variant level. Variants linked to smaller ICV were associated with increased ADHD risk. These findings can help us develop new hypotheses about biological mechanisms by which brain structure alterations may be involved in ADHD disease etiology.

Attention deficit hyperactivity disorder (ADHD) is a common, highly heritable (1, 2) neurodevelopmental disorder with a complex and heterogeneous pathophysiology. Pathways toward disease are hypothesized to be mediated by alterations in diverse brain networks (1). A recent neuroimaging mega-analysis (3) reported subtle but consistent differences in volumes of subcortical brain regions and intracranial volume (ICV) in ADHD, across diverse cohorts worldwide: compared with healthy control subjects, patients with ADHD showed decreased ICV and volumes of the nucleus accumbens, amygdala, caudate nucleus, hippocampus, and putamen. How such alterations contribute to the disease phenotype is still poorly understood. However, brain volume alterations are also present, on average, in unaffected relatives of patients with ADHD (4, 5), and both ADHD and brain volumes have high heritability (60%–70% [2, 6] and 70%–90% [7], respectively). This suggests that genetic variants underlying ADHD pathophysiology may also influence brain volume variation. Recently, the first genome-wide significant loci for ADHD were identified, and a single-nucleotide polymorphism (SNP)-based heritability of 20.16% has been reported (8). In the present study, we investigated the genetic covariance between ADHD risk and structural brain phenotypes; we set out to determine whether common genetic variants are shared between ADHD risk and brain volumes that are found to be altered in ADHD (ICV and volumes of the nucleus accumbens, amygdala, caudate nucleus, hippocampus, and putamen). These volumes were selected to focus on the most robust imaging phenotypes in ADHD (3).

Genome-wide association studies (GWASs) have identified 10 genome-wide significant loci associated with hippocampal volume (7, 9–12), eight with ICV (7, 9, 13, 14), four with putamen volume (7), and one with caudate volume (7). These variants explain only a small fraction of the heritability of these brain volumes (7, 9–11, 13). Recently, Franke and colleagues reported on a battery of statistical tools (15) to comprehensively examine genetic overlap between brain volumes and risk for brain disease at the genome-wide level and at the level of individual risk variants, using schizophrenia as an example. Here, we applied a similar set of methods to identify and dissect genetic sharing between ADHD and brain volumes implicated in ADHD based on the latest mega-analysis (3).

METHODS

This study used summary statistics of GWAS meta-analyses that were approved by local ethics committees and required informed consent (described in earlier publications [7, 8, 12, 14]).

Participant Samples

We used summary statistics data from three consortia (see the Supplementary Methods section and Table S1 in the online supplement). GWAS meta-analysis data on ADHD were from the ADHD Working Group of the Psychiatric Genomics Consortium (PGC) and the ADHD iPSYCH-SSI-Broad collaboration (20,183 case subjects, 35,191 control subjects) (8).

GWAS meta-analysis summary statistics data on ICV and volumes of the nucleus accumbens, amygdala, caudate nucleus, hippocampus, and putamen (subcortical volumes were adjusted for ICV to identify specific genetic contributions to individual volumes) were from the Enhancing Neuroimaging Genetics Through Meta-Analysis (ENIGMA) consortium (7). For the initial GWAS meta-analysis data, MRI brain scans and genome-wide genotype data were available for 11,840 subjects. During the reviewing process, we added analyses on the other two subcortical volumes (pallidum and thalamus) for which large-scale genome-wide association data were available (7); results for those analyses can be found in the extended data sheet in the online supplement.

Lastly, we obtained summary statistics of additional GWAS meta-analysis data on ICV (N=12,803 [14]) and hippocampal volume (N=13,039 [12]) from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. We did not have access to the original or preprocessed MRI scans, but rather used already existing summary statistics data based on initial GWAS meta-analyses that were performed for the different brain volumes of interest.

Before analyses were conducted, cohorts that included ADHD case subjects (N=154) were removed from the ENIGMA data (see the Supplementary Methods section in the online supplement). The summary statistics data from CHARGE were meta-analyzed with the ENIGMA data sets (see the Supplementary Methods section).

To shed some light on the potential role of IQ in the relation between ADHD and brain volume genetics, we also used summary statistics from a GWAS meta-analysis on intelligence performed in 269,867 participants (16).

Linkage Disequilibrium Score Regression (LDSR)

GWAS meta-analysis data sets underwent additional filtering (see the Supplementary Methods section). The ADHD analysis included only results from studies with samples of European (Caucasian) genetic background (case subjects, N=19,099; control subjects, N=34,194). For the ENIGMA amygdala results, the mean chi-square was too low (1.0) to reliably estimate SNP heritability using LDSR. Table 1 lists genetic correlations between brain volumes.

The analysis used a two-step procedure with the linkage disequilibrium (LD) scoring analysis package (17). An unconstrained regression estimated regression intercepts for each phenotype. Since we took measures to exclude sample overlap, we also performed the analysis with the regression intercept defined as zero (see Table S2 in the online supplement). To compute p values, standard errors were estimated using a block jackknife procedure.

SNP Effect Concordance Analysis

Postprocessing of genetic data.—To statistically compare ADHD and six brain volume GWAS meta-analyses, we used SNPs that passed quality control and filtering rules in all data sets (see the Supplementary Methods section). The clumping procedure in PLINK (18) identified an independent SNP from every LD block across the genome, providing independent SNP sets representing the total variation explained across the genome conditioned on the significance in each brain volume GWAS meta-analysis (see the Supplementary Methods section). For each of these SNP sets, we determined the corresponding ADHD GWAS meta-analysis test statistic for each independent index SNP and used these data sets for subsequent analyses.

Tests of pleiotropy and concordance.—We used SNP effect concordance analysis (SECA) (19) to determine the extent and directionality of genetic overlap between ADHD and each brain volume. Within SECA, we performed a global test of pleiotropy using a binomial test at 12 p value levels (see the Supplementary Methods section). Similarly, a two-sided Fisher's exact test estimated concordance, the agreement in SNP effect directions across two traits. We determined whether there was a significant ($p \leq 0.05$) positive or negative trend in the effect of the overlapping SNPs at each p value threshold (see the Supplementary Methods section). In total, we tested for pleiotropy and concordance between ADHD and six brain volumes. The Bonferroni-corrected significance level was set at a p value of 4.17×10^{-3} (0.05/[2×6]).

SNP Sign Test in the Top GWAS Meta-Analysis Findings

To investigate a potential accumulation of same- or opposite-direction effects of SNPs between ADHD and brain volumes, we counted the number of opposite-direction effects (as expected from the imaging results in reference 3) for top findings from the ADHD data set

in the different brain structure data sets. The ADHD GWAS meta-analysis data were clumped to define independent loci (see the Supplementary Methods section) for all variants with $p<1\times10^{-5}$ in the ADHD GWAS meta-analysis using 1KGP3v5 (20) data on European-ancestry populations as reference.

The proportion of variants with a discordant direction of effect in the individual brain GWAS meta-analyses was evaluated using a binomial test against a null hypothesis of 0.5 (i.e., chance level). This test was done for loci passing p value thresholds of 5×10^{-8} (14 LD-independent genome-wide significant SNPs), 1×10^{-6} (44 LD-independent SNPs), and 1×10^{-5} (132 LD-independent SNPs) in the ADHD GWAS meta-analysis. Details on the sign tests in the intelligence GWAS meta-analysis data (16) are provided in the Supplementary Methods section.

Weighted Meta-Analysis of ADHD and Brain Volume Data Sets

Independent of the results of the global overlap analyses, we also performed meta-analyses combining results from the ADHD GWAS meta-analysis with results from brain volume GWAS meta-analyses. We used a modified sample size–based weighting method, integrating the binary ADHD trait (ADHD risk) with the continuous trait (brain volume traits), as described in reference 8. The modified sample size–based weights were derived to account for the respective heritabilities, genetic correlation, and measurement scale of the GWAS meta-analyses (8; see also the Supplementary Methods section). For all brain volumes, we additionally performed naive meta-analyses given their low genetic correlations with ADHD risk. We set the threshold for genome-wide significance at a p value of 8.33×10^{-9} ($5\times10^{-8}/6$).

Gene-Based and Gene-Set Analyses for ADHD and Brain GWAS Meta-Analysis Data

Genome-wide summary statistics of ADHD, individual brain, and weighted combined ADHD and brain-volume GWAS meta-analysis data sets were used as input for gene-based analyses, using the Multimarker Analysis of GenoMic Annotation (MAGMA) software package, version 1.05 (21; see also the Supplementary Methods section). For the combined ADHD and brain-volume GWAS meta-analysis, only SNPs shared between ADHD and brain volume data sets were included. Overlapping significant genes ($p<2.731\times10^{-6}$) were determined and selected for further investigation (see the Supplementary Methods section).

For gene-set analyses, we used self-contained and competitive testing and tested whether genes in the neurite-outgrowth gene set (defined previously, N=45 genes; see the Supplementary Methods section and reference 22) were jointly associated with results of the weighted meta-analytic data of ADHD and ICV (see the Supplementary Methods section). Post hoc, individual genes in the set were investigated by reviewing gene test statistics of the weighted combined ADHD and ICV GWAS meta-analysis results. Genes reaching Bonferroni correction threshold (p=0.05/45=0.00111) were considered gene-wide significant. Subsequently, we reviewed gene-based associations in the ADHD GWAS meta-analysis and the ENIGMA and CHARGE ICV GWAS meta-analysis results separately.

Reciprocal Lookup of Significant GWAS Meta-Analysis Loci

Evidence for an effect of ADHD-associated SNPs on brain volume was studied through a lookup of results in the ENIGMA GWAS meta-analyses (for ICV and hippocampus volume, the ENIGMA and CHARGE GWAS meta-analysis results were used). LD-independent loci with corresponding index SNPs were obtained by clumping the summary statistics of the ADHD GWAS meta-analysis (8) (see the Supplementary Methods section). Similarly, effects of 21 independent SNPs significantly associated with brain volumes in the original publications of the brain volume GWAS meta-analyses (7, 12, 14) on ADHD risk were looked up in the ADHD GWAS meta-analysis data. If the index variant was not present in the other data set, a proxy variant was selected through LDlink (https://analysistools.nci.nih.gov/LDlink/). The Bonferroni-corrected significance levels were set at a p value of 0.000446 (0.05/[14×8]) for lookup of ADHD SNPs in brain volume GWAS meta-analysis data and at 0.002381 (0.05/21) for brain volume SNPs in ADHD GWAS meta-analysis data.

Expression Quantitative Trait Loci and Brain Gene Expression

Expression quantitative trait loci (eQTL) were examined using data from the GTEx portal (https://www.gtexportal.org/home/) (23) and the Blood eQTL Browser (http://genenetwork.nl/bloodeqtlbrowser/) (24).

We investigated the spatiotemporal expression pattern in brain tissue for selected genes using data from the Human Brain Transcriptome Project (http://hbatlas.org). We assessed mRNA expression trajectories in six regions of the developing brain and the adult brain (see the Supplementary Methods section). Gene expression over the lifespan from the spatiotemporal atlas was graphed using custom R scripts (25).

RESULTS

Comparison of Common Variant Genetic Architectures

Linkage disequilibrium score regression.—SNP-based heritability estimates for the MRI measures were consistent with previous reports (12, 14, 15) and ranged from 13.32% to 28.15% (Table 2). The amygdala mean chi-square was too small to allow a valid analysis. We observed a significant negative genetic correlation between ADHD and ICV (r_g=−0.227, p=0.00015). All other correlations were nonsignificant (Table 2; Table S2 in the online supplement shows results when using constrained intercepts).

SNP effect concordance analysis.—SECA found significant evidence of global pleiotropy for variants affecting ADHD risk for volumes of four subcortical brain regions and ICV (Table 3; see also Figure S1 in the online supplement). Discordant SNP effects for ADHD and ICV were significant, that is, variants increasing the risk for ADHD were associated with decreased ICV (p<0.001) (Table 3; see also Figure S2 in the online supplement). Evidence for concordant SNP effects reached significance for ADHD and nucleus accumbens volume (p=0.002) and for ADHD and caudate nucleus volume (p=0.004) (Table 3; see also Figure S2).

Sign tests.—Based on the phenotypic observation that patients with ADHD have, on average, smaller brain volumes compared with healthy control subjects (3), we had expected discordant rather than concordant SNP effects. As both discordant and concordant effects were seen in the SECA, we specifically determined directionality of genetic overlap between ADHD and brain volume for the top associations per trait. Thus, we zoomed in further on the most strongly associated and LD-independent SNPs and compared the signs of the regression coefficients of those top associations per trait. None of the sign tests showed a consistent direction of discordance after correction for multiple testing (see Table S3 in the online supplement). Additionally, LD-independent meta-analyzed ADHD and ICV-associated SNPs showed an overrepresentation of discordant effects in GWAS meta-analysis data for intelligence (30 of 43 SNPs, proportion=0.698, p=0.0069) (see Table S4 in the online supplement) (16).

Analyses at the Single Genetic Variant Level

Weighted SNP meta-analyses.—Based on the findings of both concordant and discordant links between ADHD and the brain volume SNPs, we performed a genome-wide search for specific genetic loci associated with both ADHD and each brain trait. We used a weighted SNP meta-analysis design allowing the combination of findings from GWAS of binary and quantitative variables (8), enabling us to specifically look for concordant effects at the level of single genetic variants; there is currently no suitable method to study discordant effects. The weighted GWAS meta-analysis for ADHD and ICV identified two significant loci of interest: chromosome 15 (*SEMA6D*) and chromosome 16 (intergenic) (Table 4, Figures 1 and 2). Four additional loci passed the study-wide threshold for genome-wide significance, but they were related to a single phenotype and did not meet criteria for cross-trait relevance (Figure 1).

We also performed weighted GWAS meta-analyses for ADHD and the four subcortical brain structures (see Figures S3–S7 in the online supplement). For amygdala volume, a naive sample size–weighted meta-analysis was performed, as no genetic correlation with ADHD had been estimated; the six novel and/or improved LD-independent genome-wide significant loci observed in these analyses are summarized in Table 4. Among those, the *SEMA6D* locus was significantly associated with ADHD and putamen volume ($p=3.62\times10^{-9}$) (see Table 4 and Figure 2; see also Figure S7 in the online supplement).

Gene-wide GWAS meta-analyses.—To maximize the power of the meta-analysis, we ran genome-wide gene-based GWAS meta-analyses in MAGMA; for gene-based results of all genes, see Tables S5–S10 in the online supplement. For the combined meta-analysis data of ADHD and ICV, three genome-wide significant genes (*MEF2C, KIZ,* and *SEMA6D*) showed stronger association in the cross-trait meta-analysis compared with the separate analyses of ADHD and ICV (see Table S11 in the online supplement). Additionally, the genome-wide significant genes *FEZF1* (amygdala), *ADD1* (caudate nucleus), and *MANBA* (hippocampus) showed increased significance in the cross-trait meta-analyses compared with the individual analyses of ADHD and brain volumes (see Tables S12–S16 in the online supplement).

Reciprocal lookup of genome-wide significant associations.—No significant associations were observed between the 14 previously identified genome-wide significant ADHD SNPs (8) and brain volumes (see Table S17 in the online supplement). Conversely, among 21 SNPs previously associated with the brain volumes (7, 12, 14), association of two ICV-linked variants (rs8756 and rs2195243) with ADHD survived correction for multiple testing ($p<0.00238$) (see Table S18 in the online supplement).

Expression Quantitative Loci and Brain Gene Expression

Previously, it was shown that many SNPs in the *SEMA6D* locus were strongly associated with expression of *SEMA6D* in fibroblasts (8, 23). Indeed, repeating this analysis for the most strongly associated variants identified by the (weighted) cross-phenotype SNP meta-analyses and the two significant variants of the reciprocal lookup using the GTEx data (23), we found rs281320 to be a significant eQTL for *SEMA6D* in transformed fibroblast tissue ($p=1.2\times10^{-20}$) (see Table S19 in the online supplement), as was rs281323 ($p=1.2\times10^{-21}$). The alternative alleles of both rs281320 and rs281323, which are associated with increased risk for ADHD and larger ICV, also increased *SEMA6D* expression (see Figure S9A, B in the online supplement). Additionally, rs12653396 was a significant eQTL for the *CTC-498M16.4* and *MEF2C* genes in brain ($p=2\times10^{-7}$) (23) and blood tissue ($p=6.53\times10^{-7}$) (24), respectively, with the disease-associated A allele being associated with increased *MEF2C* expression (see Table S19 in the online supplement). Both rs8756 and rs2195243 were eQTLs of *HMGA2* and *CCDC53*, respectively. All other top SNPs were not present in either of the two eQTL databases.

We determined mRNA expression for *SEMA6D* and *MEF2C*, the only protein-coding genes identified in the SNP-based cross-phenotype GWAS meta-analyses and four significant genes (*FEZF1, ADD1, MANBA, KIZ*) identified in the gene-based cross-trait analyses, in six brain regions of the developing and adult brain using data from the Human Brain Transcriptome Project (25). All genes are globally expressed in the developing and adult brain, with *SEMA6D* and *MEF2C* showing highest mRNA expression in prenatal periods (see Figure S8 in the online supplement).

Neurite Outgrowth Gene-Set Analysis

In an exploratory analysis, we found an association between a predefined gene set of 45 neurite outgrowth genes (22) and the meta-analytic data for the combined ADHD and ICV results using MAGMA ($p=0.00338$). It is current practice to use competitive tests, although for completeness we also report results from the self-contained analysis, which had a p value of 1.55×10^{-6}. Associations of this set with ADHD, separately, were restricted to the self-contained test ($p=5.53\times10^{-9}$); for ICV, no significant associations with the gene set were found (see Table S20 in the online supplement). In the cross-trait ADHD and ICV GWAS meta-analysis, the most strongly associated individual neurite outgrowth gene in the set was *CREB5* ($p=0.000553$); nine additional neurite outgrowth genes showed nominally significant associations (see Table S21 in the online supplement; for gene-based results of all genes, see Table S10).

DISCUSSION

In this study, we set out to investigate genetic covariance between ADHD risk and structural brain phenotypes. We found significant, though modest, genetic covariation between ADHD risk and brain volumes, both on global and gene-wide/single variant levels. On the global level, significant negative genetic correlation between ADHD and ICV was demonstrated. The direction of effect was supported by SNP effect concordance analysis. Our ICV finding was highly consistent across approaches and in the expected direction, given the previous observation that patients with ADHD have smaller ICV relative to control subjects (3). For most subcortical brain volumes, pleiotropic effects were also found. On the single variant and gene-wide levels, meta-analyses found significant loci associated with both ADHD risk and brain volumes. We identified *SEMA6D*, *KIZ*, and *MEF2C* as potential key loci contributing to both ADHD risk and ICV, and exploratory gene-set analysis revealed association of ADHD–ICV overlap with variation in neurite outgrowth genes.

A reduction of subcortical brain volumes and ICV is not unique to an ADHD diagnosis, but is also seen in depression and bipolar disorder (26, 27). However, genetic correlation between ADHD and ICV shows some specificity to this disorder, as it was not found in studies of other mental disorders, including schizophrenia (14, 15), major depressive disorder (28), and autism (14), using similar methods. On the other hand, power issues should not yet be discarded as a reason for the lack of finding genetic correlations, despite the large sample sizes, and results of a recent study found that schizophrenia and brain structure volumes share genetic risk factors using a conditional false discovery rate analysis (29). We also observed significant pleiotropy between ADHD and amygdala, caudate nucleus, hippocampus, and putamen volumes. This global genetic covariation was substantiated by local effects, which we observed in the weighted cross-phenotype meta-analyses. In addition to ICV, variation in the volumes of the caudate nucleus and putamen also showed significant genetic concordance with ADHD. However, whereas results for ICV were in line with our expectation, concordant effects for ADHD and nucleus accumbens and caudate nucleus volume were counterintuitive (ADHD patients have smaller volumes for these structures [3]), suggesting a reverse or more complex pattern of causation. It should be noted that the subcortical regions were corrected for ICV phenotypically, so that their genetic correlation was limited.

On the single variant level, we only had tools available to perform a meta-analysis by looking at concordant effects (8), and we therefore had to ignore locus-specific discordant effects. Still, the strongest association of single genetic markers was observed for ADHD and ICV, and additional associations were identified for ADHD and subcortical volumes. The weighted meta-analysis of ADHD and ICV found two potentially pleiotropic loci. One of those was *SEMA6D*, coding for the semaphorin 6D, a transmembrane molecule important for maintenance and remodeling of neuronal connections (30). Animal studies have shown that it acts as ligand for PlexinA1, which is involved in neuronal development in the spinal cord (31). Together with the gene-based cross-trait result identifying the *MEF2C* gene and the findings in the exploratory gene-set analysis, our findings suggest that neurite outgrowth dysregulation may act as a neural mediator of ADHD. Dysregulation of neurite outgrowth may pose a more general genetic risk for psychopathology, as it has been shown to be

involved not only in ADHD (22, 32) and the hyperactive/impulsive symptom domain of ADHD (33) but also in dyslexia (34) and autism (35). We also found the *SEMA6D* locus in the cross-phenotype meta-analysis for ADHD and putamen volume, even though this volume had been corrected for ICV, suggesting that genetic variation in *SEMA6D* may influence specific brain regions to varying extents. In line with our gene-set association results, a recent study using data from the UK Biobank mainly found associations between MRI measures and genes involved in brain development and plasticity (36). Since most of these genes have also been demonstrated to contribute to different psychiatric and neurodegenerative disorders (36), specificity of our findings for ADHD requires additional investigation.

Our results in this study raise a number of questions concerning the way alterations in the brain mediate etiological risk pathways in ADHD. The first question is about the role of cognitive performance in this relationship. ADHD and ICV were recently shown to be genetically correlated with intelligence (ADHD is negatively genetically correlated with IQ [r_g=−0.37, p=2.21×10^{-2}], and ICV is positively correlated with IQ [37] [r_g=0.29, p=3.44×10^{-4}]). Similarly, educational attainment is linked to both ICV (r_g=0.34, p=1.2×10^{-6}) and ADHD (r_g=−0.54, p=1.44×10^{-80}), as well as to IQ (8, 38). It may therefore be possible that the genetic link between ADHD and ICV is mediated by IQ and its proxies. We attempted to test this—in the absence of IQ and educational attainment data to correct for in the ADHD GWAS meta-analysis—using a sign test based on data from the recent large intelligence GWAS meta-analysis (16). Here, we found an overrepresentation of opposite-direction effects of ADHD–ICV SNPs in the intelligence GWAS meta-analysis data, suggesting that intelligence may indeed play a role in the ADHD–ICV overlap. However, of the 43 SNPs included in the analysis, only 15 (34.8%) were nominally significantly associated with intelligence, suggesting that the genetic link between ADHD and ICV is additionally driven by intelligence-independent effects. More in-depth research will be needed to fully understand the role of intelligence in the ADHD–ICV overlap in the future; it may occur upstream and/or downstream of our correlation finding. Second, the degree of sharing observed was statistically modest. At first sight, this seems to be inconsistent with the general hypothesis that ADHD is a genetic-based braind is order. However, there are a number of possible explanations for this modest sharing. We examined brain structure at a gross anatomical scale; compared with more precise methods, such as voxel-based or surface-based morphometry, atlas-based brain segmentations may be too coarse to identify subtler volumetric differences. Notably, for the type of imaging genetics analyses described here, we were strongly dependent on the availability of GWAS data for brain phenotypes. These GWAS data have to be derived from large-scale studies to allow sufficiently powered analyses. Such data are, so far, available only for subcortical volumes and ICV, published by the ENIGMA and CHARGE consortia. The sample sizes of the few voxel-wise GWASs available to date are not large enough to offer sufficient statistical power for the genome-wide approaches presented here. Moreover, it may be more informative to study structural and functional connectivity measures. In addition, as pointed out previously (15), the limited SNP heritability of subcortical brain volumes further challenges the identification of genetic overlap, and more highly powered studies of brain phenotypes may lead to higher estimates of overlap. Also, the field may advance by applying more

sophisticated imaging approaches to imaging genetics studies, such as redefining imaging phenotypes through dimension reduction approaches (39). Finally, it is also possible that some of ADHD's association with reduced brain volumes is driven by environmental effects, either independently or in interaction with genetic factors (1).

Previous brain imaging genetics studies in ADHD have mainly focused on single genetic variants and have been hampered by limited sample sizes (40). This study combined the largest data sets available to investigate the genetic overlap between ADHD and brain volumes by using a complementary battery of statistical methods. Nevertheless, some limitations apply. First, this study focused on a limited set of mainly subcortical MRI measures, and future work should be extended to cortical regions and connectivity measures, once large-scale GWAS meta-analysis becomes available (4, 41). To support highly sophisticated imaging genetics analyses, which can provide granular information on specific circuits of relevance, there is also an increasing need for large-scale imaging cohorts with raw imaging and genetic data that allow maximal flexibility in the application of analytic methods. Second, for the cross-phenotype GWAS meta-analysis, we used a recently described weighted meta-analysis method (8). However, we observed that with low and moderate genetically correlated phenotypes, the association signals generally did not improve over a naive meta-analysis performed without adding additional weights (see Figure S10 and Table S22 in the online supplement). In addition, we could only investigate concordant SNP effects with this method. Third, generalization of our findings to other ethnicities should be assessed in future studies. Fourth, it is possible that this study underestimated genetic correlations, as we did not take into account the known role of rare and structural variants in the genetic architecture of ADHD (42, 43). Future studies investigating heritability and genetic correlation could also benefit from including variants with low minor allele frequency and in low-LD regions, which may reveal stronger relationships between ADHD and brain volumes.

To our knowledge, this is the first study to show significant global and single gene/variant level genetic correlations derived from polygenic overlap between ADHD and brain volumes. The modest genetic overlap between ADHD and variation in brain volumes is consistent with models implicating alterations in brain structure in ADHD-related genetic risk pathways and provides new hypotheses about neurobiological mechanisms involved in ADHD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The Enhancing Neuroimaging Genetics Through Meta-Analysis (ENIGMA) Consortium provided summary statistics of the consortium findings. The original publication of those findings as well as the list of contributing samples and authors may be found on the ENIGMA web site (http://enigma.ini.usc.edu). The Neurology Working Group of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium also contributed with an independent set of summary statistics of consortium findings. The lists of contributing cohorts and authors may be found in previous publications of the CHARGE consortium, as listed on the consortium’s web site (http://www.chargeconsortium.com/). The ADHD working group of the Psychiatric Genomics Consortium (PGC) and the iPSYCH-SSI-Broad collaboration ADHD Working Group contributed with an independent set of summary of consortium findings. The data and a complete list of contributing samples and people can be obtained from the PGC web site’s download page (https://www.med.unc.edu/pgc/results-and-downloads).

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REFERENCES

1. Faraone SV, Asherson P, Banaschewski T, et al.: Attention-deficit/hyperactivity disorder. Nat Rev Dis Primers 2015; 1:15020 [PubMed: 27189265]
2. Faraone SV, Perlis RH, Doyle AE, et al.: Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 2005; 57: 1313–1323 [PubMed: 15950004]
3. Hoogman M, Bralten J, Hibar DP, et al.: Subcortical brain volume differences in participants with attention deficit hyperactivity disorder in children and adults: a cross-sectional mega-analysis. Lancet Psychiatry 2017; 4:310–319 [PubMed: 28219628]
4. Bralten J, Greven CU, Franke B, et al.: Voxel-based morphometry analysis reveals frontal brain differences in participants with ADHD and their unaffected siblings. J Psychiatry Neurosci 2016; 41: 272–279 [PubMed: 26679925]
5. Greven CU, Bralten J, Mennes M, et al.: Developmentally stable whole-brain volume reductions and developmentally sensitive caudate and putamen volume alterations in those with attention-deficit/hyperactivity disorder and their unaffected siblings. JAMA Psychiatry 2015; 72:490–499 [PubMed: 25785435]
6. Larsson H, Chang Z, D’Onofrio BM, et al.: The heritability of clinically diagnosed attention deficit hyperactivity disorder across the lifespan. Psychol Med 2014; 44:2223–2229 [PubMed: 24107258]
7. Hibar DP, Stein JL, Renteria ME, et al.: Common genetic variants influence human subcortical brain structures. Nature 2015; 520: 224–229 [PubMed: 25607358]
8. Demontis D, Walters RK, Martin J, et al.: Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat Genet 2019; 51:63–75 [PubMed: 30478444]
9. Stein JL, Medland SE, Vasquez AA, et al.: Identification of common variants associated with human hippocampal and intracranial volumes. Nat Genet 2012; 44:552–561 [PubMed: 22504417]
10. Bis JC, DeCarli C, Smith AV, et al.: Common variants at 12q14 and12q24 are associated with hippocampal volume. Nat Genet 2012; 44: 545–551 [PubMed: 22504421]
11. Melville SA, Buros J, Parrado AR, et al.: Multiple loci influencing hippocampal degeneration identified by genome scan. Ann Neurol 2012; 72:65–75 [PubMed: 22745009]

12. Hibar DP, Adams HHH, Jahanshad N, et al.: Novel genetic loci associated with hippocampal volume. Nat Commun 2017; 8:13624 [PubMed: 28098162]
13. Ikram MA, Fornage M, Smith AV, et al.: Common variants at 6q22 and 17q21 are associated with intracranial volume. Nat Genet 2012; 44: 539–544 [PubMed: 22504418]
14. Adams HH, Hibar DP, Chouraki V, et al.: Novel genetic loci underlying human intracranial volume identified through genome-wide association. Nat Neurosci 2016; 19:1569–1582 [PubMed: 27694991]
15. Franke B, Stein JL, Ripke S, et al.: Genetic influences on schizophrenia and subcortical brain volumes: large-scale proof of concept. Nat Neurosci 2016; 19:420–431 [PubMed: 26854805]
16. Savage JE, Jansen PR, Stringer S, et al.: Genome-wide association meta-analysis in 269,867 individuals identifies new genetic and functional links to intelligence. Nat Genet 2018; 50:912–919 [PubMed: 29942086]
17. Bulik-Sullivan BK, Loh PR, Finucane HK, et al.: LD score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet 2015; 47:291–295 [PubMed: 25642630]
18. Purcell S, Neale B, Todd-Brown K, et al.: PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559–575 [PubMed: 17701901]
19. Nyholt DR: SECA: SNP effect concordance analysis using genome-wide association summary results. Bioinformatics 2014; 30:2086–2088 [PubMed: 24695403]
20. Auton A, Brooks LD, Durbin RM, et al.: A global reference for humangenetic variation. Nature 2015; 526:68–74 [PubMed: 26432245]
21. De Leeuw CA, Mooij JM, Heskes T, et al.: MAGMA: generalized gene-set analysis of GWAS data. PLOS Comput Biol 2015; 11:e1004219 [PubMed: 25885710]
22. Poelmans G, Pauls DL, Buitelaar JK, et al.: Integrated genome-wide association study findings: identification of a neurodevelopmental network for attention deficit hyperactivity disorder. Am J Psychiatry 2011; 168:365–377 [PubMed: 21324949]
23. GTEx Consortium: The Genotype-Tissue Expression (GTEx) project. Nat Genet 2013; 45:580–585 [PubMed: 23715323]
24. Westra HJ, Peters MJ, Esko T, et al.: Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 2013; 45:1238–1243 [PubMed: 24013639]
25. Kang HJ, Kawasawa YI, Cheng F, et al.: Spatio-temporal transcriptome of the human brain. Nature 2011; 478:483–489 [PubMed: 22031440]
26. Hibar DP, Westlye LT, van Erp TG, et al.: Subcortical volumetric abnormalities in bipolar disorder. Mol Psychiatry 2016; 21:1710–1716 [PubMed: 26857596]
27. Schmaal L, Veltman DJ, van Erp TG, et al.: Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry 2016; 21: 806–812 [PubMed: 26122586]
28. Wigmore EM, Clarke T-K, Adams MJ, et al.: Do regional brain volumes and major depressive disorder share genetic architecture: a study in Generation Scotland (N=19,762), UK Biobank (N=24,048) and the English Longitudinal Study of Ageing (N=5,766). bioRxiv, 12 8, 2016 (doi: 10.1101/059352)
29. Smeland OB, Wang Y, Frei O, et al.: Genetic overlap between schizophrenia and volumes of hippocampus, putamen, and intracranial volume indicates shared molecular genetic mechanisms. Schizophr Bull 2018; 44:854–864 [PubMed: 29136250]
30. Qu X, Wei H, Zhai Y, et al.: Identification, characterization, and functional study of the two novel human members of the semaphorin gene family. J Biol Chem 2002; 277:35574–35585 [PubMed: 12110693]
31. Yoshida Y, Han B, Mendelsohn M, et al.: PlexinA1 signaling directs the segregation of proprioceptive sensory axons in the developing spinal cord. Neuron 2006; 52:775–788 [PubMed: 17145500]
32. Mooney MA, McWeeney SK, Faraone SV, et al.: Pathway analysis in attention deficit hyperactivity disorder: an ensemble approach. Am J Med Genet B Neuropsychiatr Genet 2016; 171:815–826 [PubMed: 27004716]

33. Bralten J, Franke B, Waldman I, et al.: Candidate genetic pathways for attention-deficit/hyperactivity disorder (ADHD) show association to hyperactive/impulsive symptoms in children with ADHD. J Am Acad Child Adolesc Psychiatry 2013; 52:1204–1212.e1 [PubMed: 24157394]
34. Poelmans G, Buitelaar JK, Pauls DL, et al.: A theoretical molecular network for dyslexia: integrating available genetic findings. Mol Psychiatry 2011; 16:365–382 [PubMed: 20956978]
35. Poelmans G, Franke B, Pauls DL, et al.: AKAPs integrate genetic findings for autism spectrum disorders. Transl Psychiatry 2013; 3: e270 [PubMed: 23756379]
36. Elliott L, Sharp K, Alfaro-Almagro F, et al.: The genetic basis of human brain structure and function: 1,262 genome-wide associations found from 3,144 GWAS of multimodal brain imaging phenotypes from 9,707 UK Biobank participants. bioRxiv, 8 21, 2017 (doi:10.1101/178806)
37. Sniekers S, Stringer S, Watanabe K, et al.: Genome-wide association meta-analysis of 78,308 individuals identifies new loci and genes influencing human intelligence. Nat Genet 2017; 49:1107–1112 [PubMed: 28530673]
38. Okbay A, Beauchamp JP, Fontana MA, et al.: Genome-wide association study identifies 74 loci associated with educational attainment. Nature 2016; 533:539–542 [PubMed: 27225129]
39. Fan CC, Smeland OB, Schork AJ, et al.: Beyond heritability: improving discoverability in imaging genetics. Hum Mol Genet 2018; 27 (R1):R22–R28 [PubMed: 29522091]
40. Klein M, Onnink M, van Donkelaar M, et al.: Brain imaging genetics in ADHD and beyond: mapping pathways from gene to disorder at different levels of complexity. Neurosci Biobehav Rev 2017; 80: 115–155 [PubMed: 28159610]
41. Wu ZM, Bralten J, Cao QJ, et al.: White matter microstructural alterations in children with ADHD: categorical and dimensional perspectives. Neuropsychopharmacology 2017; 42:572–580 [PubMed: 27681441]
42. Demontis D, Lescai F, Børglum A, et al.: Whole-exome sequencing reveals increased burden of rare functional and disruptive variants in candidate risk genes in individuals with persistent attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 2016; 55: 521–523 [PubMed: 27238071]
43. Williams NM, Zaharieva I, Martin A, et al.: Rare chromosomal deletions and duplications in attention-deficit hyperactivity disorder: a genome-wide analysis. Lancet 2010; 376:1401–1408 [PubMed: 20888040]

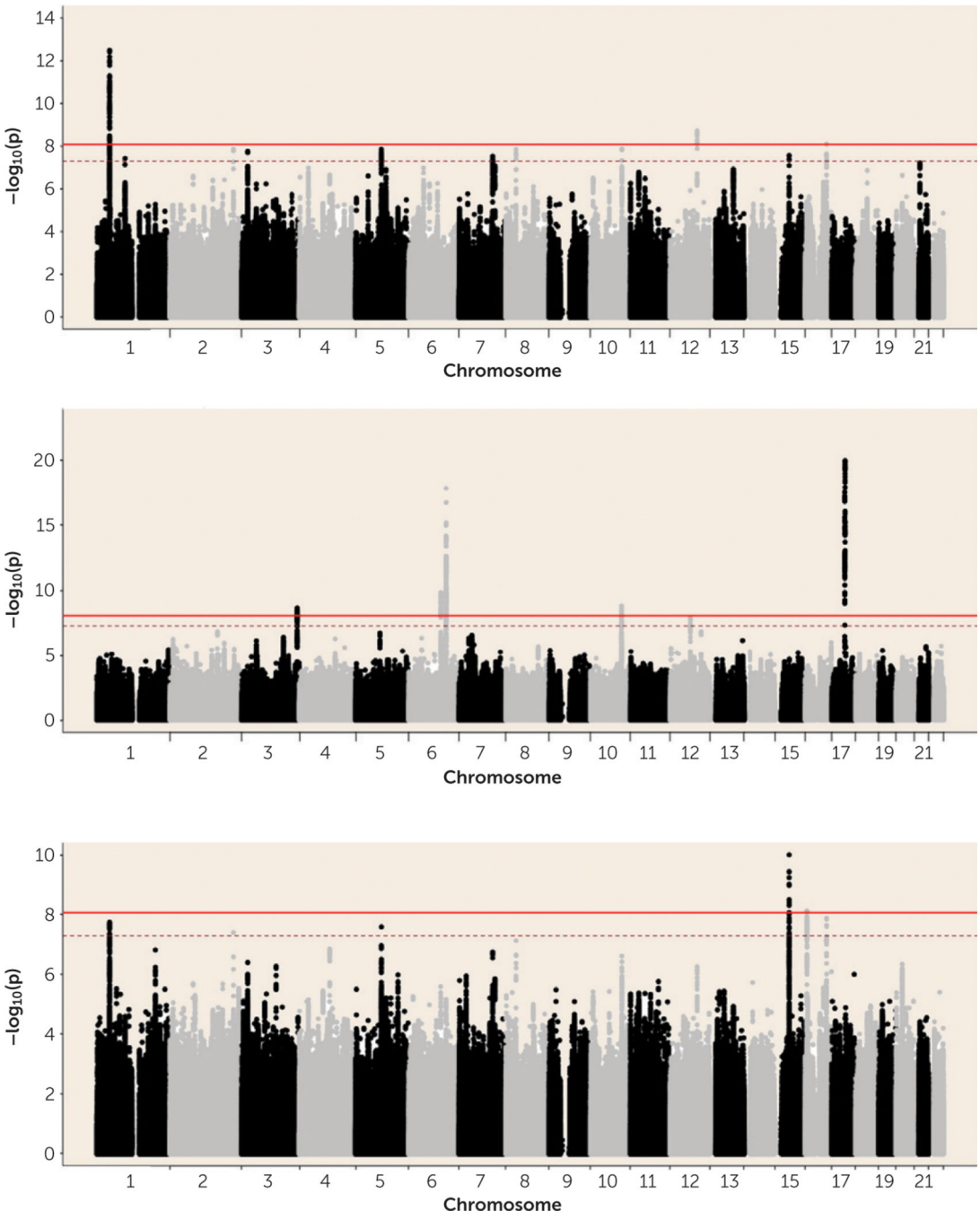

FIGURE 1. Common genetic variants associated with ADHD, intracranial volume (ICV), and the combined analysis of ADHD and ICV[a]

A. ADHD GWAS Meta-Analysis (PGC and iPSYCH Data)

B. ICV GWAS Meta-Analysis (ENIGMA and CHARGE Data)

C. Combined ADHD and ICV Weighted GWAS Meta-Analysis

[a]Shown here are Manhattan plots, in which every point represents a single genetic variant plotted according to its genomics position (x-axis) and its $-\log_{10}(p)$ value for association with the respective trait (y-axis). The solid red line represents the study-wide genome-wide significance of $p<8.33\times10^{-9}$, and the dashed red line represents the genome-wide

significance of $p<5\times10^{-8}$. ADHD=attention deficit hyperactivity disorder; CHARGE=Cohorts for Heart and Aging Research in Genomic Epidemiology; ENIGMA=Enhancing Neuroimaging Genetics Through Meta-Analysis; GWAS=genome-wide association study; PGC=Psychiatric Genomics Consortium.

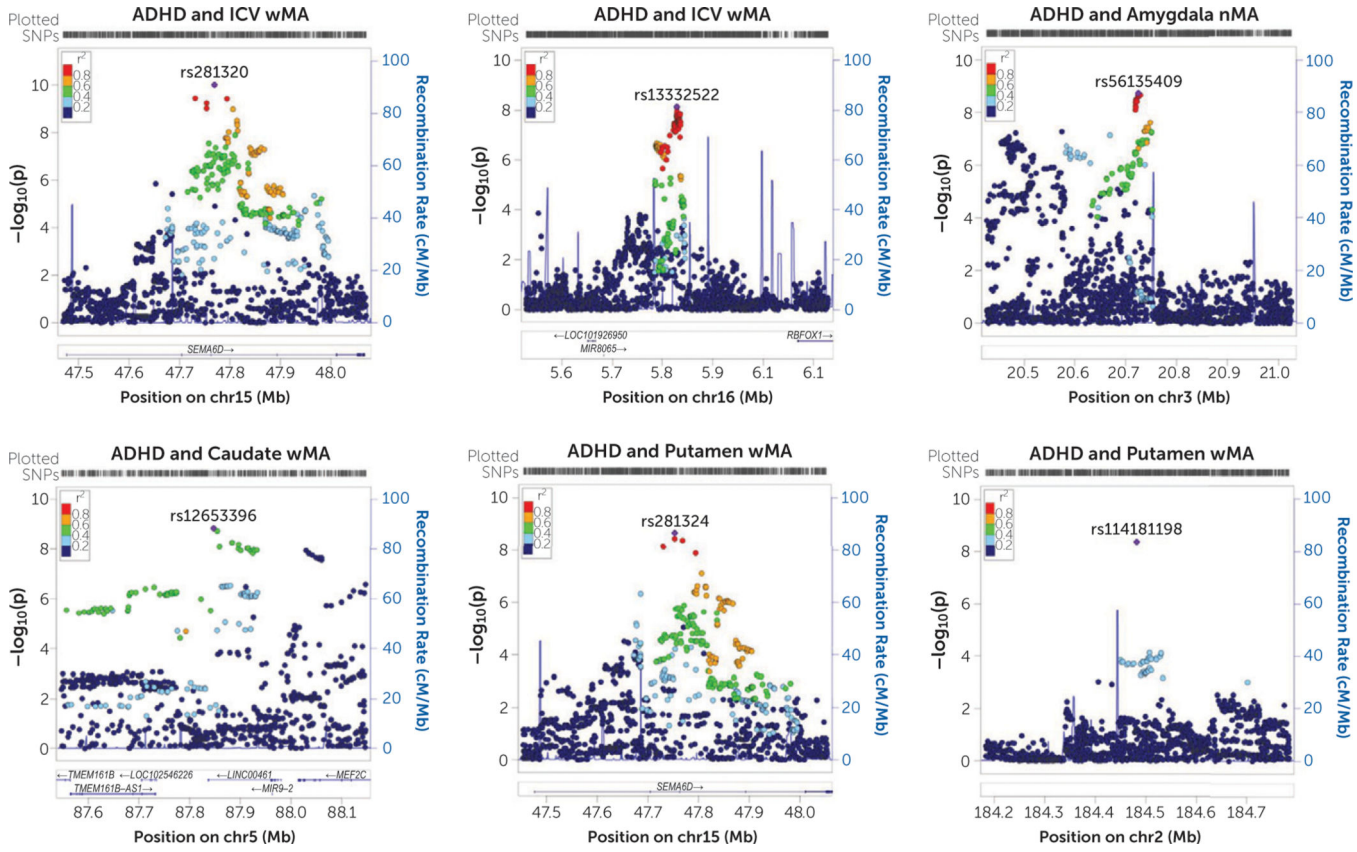

FIGURE 2. Regional association of genome-wide significant loci of ADHD and brain volume GWAS meta-analyses[a]

[a] For each panel, zoomed-in association plots (±300 kb from the top SNP, indexed by purple diamond) are shown. Plots are zoomed to highlight the genomic region that likely harbors the causal variant(s). ADHD=attention deficit hyperactivity disorder; nMA=naive meta-analysis of ADHD and brain volume; wMA=weighted meta-analysis of ADHD and brain volume; SNP=single-nucleotide polymorphism.

TABLE 1.

Genetic correlations between brain volumes[a]

	Intracranial Volume		Nucleus Accumbens		Caudate Nucleus		Hippocampus	
Trait	**r_g**	**p**	**r_g**	**p**	**r_g**	**p**	**r_g**	**p**
Intracranial volume[b]								
Nucleus accumbens	0.02727	0.8353						
Caudate nucleus	0.1158	0.2323	0.4191	0.007684				
Hippocampus[b]	0.2559	**0.001704**	0.1891	0.2218	−1.725	0.1991		
Putamen	0.08746	0.306	0.4357	**1.67×10^{-3}**	0.175	0.173	0.08832	0.3802

[a]The amygdala mean chi-square was too small to allow a valid analysis (N=11,757). r_g=genetic correlation.

[b]Using GWAS meta-analysis summary statistics from the meta-analysis of ENIGMA and CHARGE cohorts. Genetic correlations were estimated by using free intercepts. In accordance with the number of tests performed, we set a Bonferroni-corrected significance level at a p value of 0.005 (0.05/10); p values in boldface are significant after Bonferroni correction. CHARGE=Cohorts for Heart and Aging Research in Genomic Epidemiology; ENIGMA=Enhancing Neuroimaging Genetics Through Meta-Analysis; GWAS=genome-wide association study.

TABLE 2.

SNP heritability analyses for MRI brain volumes and genetic correlation with ADHD[a]

Brain Region	N	Heritability	SE	Genetic Correlation With ADHD	SE	Z	p
Nucleus accumbens	11,709	0.1332	0.0518	0.005558	0.09487	0.05858	0.9533
Caudate nucleus	11,772	0.2456	0.0455	−0.06426	0.07321	−0.8778	0.3801
Hippocampus[b]	24,704	0.1418	0.0286	−0.02354	0.06902	−0.3411	0.733
Intracranial volume[b]	24,024	0.2318	0.0325	−0.2266	0.05989	−3.784	**0.0001543**
Putamen	11,646	0.2815	0.056	0.006433	0.07077	0.09089	0.9276
Hippocampus, ENIGMA only	11,665	0.1363	0.0488	−0.04202	0.09965	−0.4217	0.6733
Hippocampus, CHARGE only	13,039	0.16	0.042	−0.0126	0.0832	−0.1516	0.8795
Intracranial volume, ENIGMA only	11,221	0.1745	0.0461	−0.2348	0.09452	−2.485	0.01296
Intracranial volume, CHARGE only	12,803	0.283	0.0466	−0.2305	0.071	−3.2479	0.0012

[a]The amygdala mean chi-square was too small to allow a valid analysis (N=11,757).

[b]Using GWAS meta-analysis summary statistics from the meta-analysis of ENIGMA and CHARGE cohorts. Heritability and genetic correlation were estimated by using free intercepts. In accordance with the number of tests performed, we set a Bonferroni-corrected significance level at a p value of 0.0083 (0.05/6); the p value in boldface is significant after Bonferroni correction. ADHD=attention deficit hyperactivity disorder; CHARGE=Cohorts for Heart and Aging Research in Genomic Epidemiology; ENIGMA=Enhancing Neuroimaging Genetics Through Meta-Analysis; GWAS=genome-wide association study.

TABLE 3.

Results of pleiotropy and concordance test of SNP effect concordance analysis, with brain volume GWAS meta-analysis conditioned on ADHD GWAS meta-analysis[a]

	Pleiotropy		Concordance		
Brain Volume	**p**	**95% CI**	**p**	**95% CI**	**Direction of SNP Effects**
Nucleus accumbens	0.034	0.0244, 0.0471	**0.002**	0.000548, 0.00726	Concordant
Amygdala	**<0.001**	5.12×10^{-5}, 0.00564	0.006	0.00275, 0.013	Discordant
Caudate nucleus	**<0.001**	5.12×10^{-5}, 0.00564	**0.004**	0.00156, 0.0102	Concordant
Hippocampus[b]	**0.002**	0.000548, 0.00726	1	0.996, 1	
Intracranial volume[b]	**<0.001**	5.12×10^{-5}, 0.00564	**<0.001**	5.12×10^{-5}, 0.00564	Discordant
Putamen	**<0.001**	5.12×10^{-5}, 0.00564	0.01	0.00544, 0.0183	Concordant
Hippocampus, ENIGMA only	0.005	0.00214, 0.0116	1	0.996, 1	
Intracranial volume, ENIGMA only	**<0.001**	5.12×10^{-5}, 0.00564	**<0.001**	5.12×10^{-5}, 0.00564	Discordant

[a]The p values and confidence intervals were obtained based on 1,000 permutations.

[b]Using GWAS meta-analysis summary statistics from the meta-analysis of ENIGMA and CHARGE cohorts. In accordance with the number of tests performed, we set a Bonferroni-corrected significance level at a p value of 0.00416 (0.05/[2×6]); p values in boldface are significant after Bonferroni correction. ADHD=attention deficit hyperactivity disorder; CHARGE=Cohorts for Heart and Aging Research in Genomic Epidemiology; ENIGMA=Enhancing Neuroimaging Genetics Through Meta-Analysis; GWAS=genome-wide association study; SNP=single-nucleotide polymorphism.

TABLE 4.

Results of weighted meta-analyses of ADHD and brain volumes showing novel or improved independent genome-wide significant loci[a]

								Direction of Association		ADHD Effect		Brain Volume Effect	ENIGMA2 Data		
Brain Trait	**Chr**	**Position**	**Gene**	**SNP**	**A1**	**Z-Score**	**p**	**ADHD**	**Brain**	**Z-Score**	**Odds Ratio**	**Z-Score**	**Effect**	**SE**	**Variance Explained (%)**
Intracranial volume	15	47769424	*SEMA6D*	rs281320[b]	T	−6.47	9.95×10^{-11}	–	–	−5.55	0.93	−3.33	−4772.06	1923.44	0.05
Intracranial volume	16	5829204	Intergenic	rs13332522[c]	C	5.78	7.43×10^{-9}	+	+	4.62	1.07	3.51	2986.55	2174.06	0.017
Amygdala	3	20725016	Intergenic	rs56135409[b]	A	−6.01	1.85×10^{-9}	–	–	−5.35	0.93	−2.75	−6.86	2.50	0.07
Caudate nucleus	5	87847273	*LINC00461*	rs12653396[c]	A	5.38	1.46×10^{-9}	+	+	5.38	1.08	2.95	14.87	5.04	0.07
Putamen	15	47754027	*SEMA6D*	rs281323[b]	A	5.90	3.62×10^{-9}	+	+	5.48	1.08	2.21	13.62	6.16	0.04
Putamen	2	184483159	Intergenic	rs114181198[c]	A	5.13	4.28×10^{-9}	+	+	5.09	1.13	3.26	36.07	11.06	0.09

[a]Results for six genome-wide significant index SNPs identified in the GWAS meta-analyses of ADHD and brain volumes. The allele (A1) for the effect of the Z-score is given. Direction gives information about the direction of the association in the two samples, "+" indicates that A1 increases risk or volume, and "−" indicates that A1 decreases risk or volume. The threshold for genome-wide significance was set at $p<8.33\times10^{-9}$ (5×10^{-8}/6 traits). ADHD=attention deficit hyperactivity disorder; ENIGMA2=Enhancing Neuroimaging Genetics Through Meta-Analysis, GWAS meta-analysis of subcortical volumes; GWAS=genome-wide association study; SNP=single-nucleotide polymorphism.

[b]This locus improved by one order of magnitude after the meta-analysis.

[c]This locus is a new genome-wide significant locus in the ADHD and brain volume meta-analysis. The odds ratio for the ADHD effect is based on initial Psychiatric Genomics Consortium (PGC) and iPSYCH GWAS meta-analysis that was used as input for the weighted meta-analysis. Effect sizes are given in units of mm^3 per effect allele. Results are provided for the ENIGMA2 cohort only. The variance explained gives the percentage variance explained by a given SNP after correcting for covariates (see the Supplementary Methods section in the online supplement for additional details).